AN *ISOSPORA* OF CIVET CATS

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PLATE V

An *Isospora* was studied in three heavily infected civet cats, *Viverra civetta*—two young animals in which the infection proved fatal, and one adult which was killed for pathological examination.

The animals passed blood and mucus and a large number of oöcysts in their faeces.

The parasite was studied in scrapings of infected mucosa, in sections and in smears. Smears were prepared in the following way : a portion of infected gut was gently washed in water to remove débris, the mucosa was then removed with a scalpel and gently rubbed on the surface of a slide ; prepared in this way, the relationship between the parasite and the epithelial cells is in many instances hardly disturbed. Some of the smears were fixed in Schaudinn's fluid and stained with iron haematoxylin ; others were fixed in methyl alcohol and stained with Giemsa's stain. If the débris is carefully washed away from the infected mucosa, Giemsa's stain gives good results.

DESCRIPTION OF THE PARASITE IN THE HOST

Development is confined to the epithelium of the lower half of the small intestine. Although trophozoites penetrate into the tissue they do not undergo further development there ; their nucleus breaks up into granules and they eventually disintegrate, or they are occasionally engulfed in the lymphocytes (fig. 9).

The merozoites vary in size from 4·0μ to 9·6μ in length by 0·8μ to 2·4μ in thickness. This large variation depends on the number

of merozoites in the schizonts, on the size of the schizonts, and partly on individual variation. Thus in abnormal schizonts containing four merozoites (fig. 19) these may reach 9·6μ in length by 2·4μ in breadth, and on the other hand, in schizonts containing more than eight merozoites (fig. 22) they may be as small as 4μ in length by 0·8μ in breadth. In normal schizonts containing eight merozoites which are by far the commonest (figs. 20 and 21) variations in size occur (4·8μ to 6·2μ in length by 1μ to 1·8μ in breadth). There is no evidence that the large merozoites found in schizonts are differentiated for the sexual cycle. Probably the size of the merozoites in this particular parasite is determined only by the space available for growth inside the schizont and has no sexual significance. The merozoites inside the schizont are usually slightly bent and are pointed at one extremity.

The nucleus of the merozoite is relatively large ; it has a distinct nuclear membrane and contains a large karyosome which may lie in the centre of the nucleus or it may be attached to a portion of the nuclear membrane.

Trophozoites are found inside vacuoles in the infected cell, the pointed end of the parasite being attached to a point in the wall of the vacuole (figs. 1 and 2). Double infection of cells was frequently seen and occasionally three merozoites were found parasitising one cell.

In cases of multiple infection, two trophozoites may be found in one vacuole (fig. 16), or each parasite may lie in its own vacuole (fig. 17).

The trophozoites grow inside their vacuoles and are usually gregariniform in shape. The variations found in the shapes of the trophozoites are shown in figs. 3 to 8.

When the trophozoite has attained a size of 9·6μ to 11·1μ in length by 3·2μ to 3·7μ in its thickest part, nuclear division takes place. This is accomplished in the following manner :—The karyosome divides by simple fission, and the two daughter karyosomes move to opposite ends of the nucleus ; between them a spindle stretches, on which two plates of chromosomes form ; these move towards the daughter karyosomes and the nuclear membrane then divides by constriction between the two karyosomes, two daughter nuclei being formed. The schizont maintains its gregarine form

till it contains eight nuclei ; it then becomes round, the protoplasm segments and eight merozoites are formed. The schizont may or may not contain a residual body. Abnormal schizonts are found which contain more or less than eight merozoites (figs. 18, 19, 22). Schizonts vary from 10μ in diameter (containing four merozoites) to 18·4μ. Normal schizonts containing eight merozoites are generally about 16μ in diameter.

MICROGAMETOCYTES

As previously stated it was not found possible to distinguish the merozoites destined to become microgametocytes, but after several nuclear divisions have occurred, it is easy to distinguish the microgametocyte from the schizont, for it tends to become round or elliptical when it is only 6μ in diameter and it then contains about thirty small round nuclei (fig. 30).

When the growing microgametocyte becomes sufficiently large it is possible to see that nuclear division takes place by mitosis (fig. 32). A stage is reached when nuclear division ceases and the microgametocyte then contains a large number of irregular compact nuclei (fig. 33) ; these become round and from each nucleus a small rod of chromatin protrudes (fig. 34). The nucleus then becomes falciform and finally microgametocytes develop (fig. 35).

The completely developed microgametocyte varies from 16μ to 24·7μ in diameter and contains one, or more commonly two residual bodies round which the microgametes are grouped. There are usually about two hundred microgametes in one fully developed microgametocyte. The microgamete has two flagella and measures about 10μ from the tip of one flagella to the other.

THE MACROGAMETE

The young macrogamete is easily recognisable, for it becomes spherical early in its development, when only 7μ in diameter. At this stage it is very striking, because of its large round nucleus which takes up half the diameter of the parasite (figs. 23 and 24). The nucleus of the young macrogamete is the same in size as well as structure as that of the mature macrogamete. Deeply staining

granules appear in the protoplasm round the nuclear membrane and as the parasite grows, these granules diminish in number and are disseminated throughout the protoplasm. The nucleus of the host cell becomes attached to and often drawn over the vacuole containing the macrogamete (figs. 26 and 28), but this is not absolutely typical of the macrogamete, for it is also occasionally seen in microgametocytes and schizonts.

The protoplasm of the macrogamete consists of a very fine mesh containing small refractile granules. The mature macrogamete attains almost the same size as the oöcyst. Macrogametes were found to be about thirty times as numerous as microgametocytes.

Fertilisation takes place inside the host cell. As many as nine or ten microgametes were observed inside one macrogamete (fig. 29).

After fertilisation the hard wall of the oöcyst is formed. The protoplasm of the oöcyst contains coarser granules than the macrogamete. In addition to the numerous small refractile granules, the protoplasm contains from one to five larger refractile bodies and these are extruded during the first division of the oöcyst (figs. 36 and 37) forming an oöcystic residue which disappears before development of the oöcyst is completed. The first division takes place inside the invaded epithelial cell or in the gut. Oöcysts containing two sporoblasts are found in the gut with the remains of the nucleus and protoplasm of the invaded cell attached to them. No further development takes place inside the host and the oöcysts containing two sporoblasts are passed in the faeces.

A number of oöcysts are passed with the protoplasm unsegmented but they are probably non-fertilised macrogametes which have become encysted, for they do not appear to develop further outside the body of the host.

DEVELOPMENT OUTSIDE THE HOST

After twenty-four hours the sporoblasts have a sporocyst round them and become spores, each spore containing two masses of protoplasm and a residual body. The spore measures 12·5μ to 15·2μ in length by 8μ to 11μ in breadth ; the residual body at this stage measures 6μ by 10μ.

After three days' development is completed, each spore then contains four sporozoites and a residual body ; the sporozoites are sickle-shaped and measure 9·5μ to 11·2μ in length by 2·8μ in their thickest part ; the residual body is more diffused than in the previous stage, so that it is impossible to give definite measurements. After standing for two or three weeks, the residual body in the spore is reduced to a few granules.

The oöcysts vary in size from 19μ to 27·5μ in length by 15·2μ to 24·7μ in breadth, the commonest size being 22·8μ by 19μ, and the extremes 15·2μ by 19μ and 24·7μ by 27·5μ being only two per cent. of the total number measured. They thus approximate in size to, but are slightly larger than *Isospora rivoltae*, Grassi, 1879 (15μ to 20μ by 20μ to 24μ) in English dogs (Wenyon, 1923).

IDENTITY OF THE PARASITE

The oöcysts are approximately the same size as *Isospora rivoltae*, Grassi, 1879 ; but the *Isospora* of civet cats has certain features which distinguish it from the former.

(1) Its oöcysts are passed containing two sporoblasts whereas in *I. rivoltae* the oöcysts are passed unsegmented.

(2) It was not found possible to infect cats and kittens, and young dogs by feeding with heavily infected material. Two cats, three kittens and two young dogs were used for the experiment, and they failed to become infected within an observation period of twenty-eight days.

The *Isospora* of civet cats is therefore probably not the same species as *Isospora rivoltae* and I propose the name *Isospora viverrae*, n.sp., for the parasite.

PATHOLOGY OF THE INFECTION

On section, the infected parts of the small intestine showed that in the basal parts of the villi few cells were infected, whereas the distal parts were heavily infected. A similar observation was made by Wenyon in the case of *Isospora felis*, Wenyon, 1923. In many villi the distal part was entirely denuded of the epithelium. The sub-epithelial tissue in infected areas was markedly hyperaemic.

In spite of the heavy infection and the changes of the sub-epithelial tissues no ulceration was observed. In this connection it is interesting to note that during 1922 and 1923 about thirty specimens of *Viverra civetta* were examined in the Sir A. L. Jones Laboratory, in Freetown, and in no case was ulceration of the small intestine noted. It seems probable, therefore, that regeneration takes place from the epithelium of the basal and comparatively unaffected portions of the villi.

An interesting fact was observed, namely, that in heavily infected parts of the small intestine islands were found which on serial section appear to be free from infection. It is hardly likely that the cells of these portions surrounded by heavily infected areas were not subjected to invasion by merozoites. A more feasible explanation is, that these islands of healthy mucosa had been infected and the villi denuded of epithelium distally ; regeneration from the healthy epithelium situated at the base of the villi then took place and the regenerated areas became immune to further infection, i.e., we are dealing with a case of local and acquired immunity to a protozoon.

In the two young animals, numerous schizonts as well as sexual forms were found, whereas in the adult animal schizonts were comparatively few and were only found after prolonged search ; it is probable, therefore, that the adult animal was on the eve of a spontaneous cure in spite of a heavy infection.

SUMMARY AND CONCLUSIONS

An *Isospora* was studied in three heavily infected civet cats.

The asexual and sexual cycles within the host are described.

Oöcysts containing two sporoblasts were found in the intestine and in fresh faeces.

The oöcysts measured 19μ to 27·5μ in length by 15·2μ to 24·7μ in breadth.

The development of the oöcysts outside the body of the host is described.

Cats and dogs fed on heavily infected material failed to become infected within an observation period of twenty-eight days.

The name *Isospora viverrae*, n.sp., is proposed for the *Isospora* of civet cats.

REFERENCES

Brumpt, E. (1922). Précis de Parasitologie. Paris. pp. 127-135.

Castellani, A., and Chalmers, A. J. (1919). Manual of Tropical Medicine. 3rd ed., pp. 471-477.

Dobell, C., and O'Connor, F. W. (1921). The Intestinal Protozoa of Man. pp. 94-105.

Fantham, H. B., Stephens, J. W. W., and Theobald, F. V. (1916). The Animal Parasites of Man, pp. 135-150.

Knuth, P., and du Toit, P. J. (1921). Tropenkrankheiten der Haustiere, pp. 523-535 (Mense, *Handbuch der Tropenkrankheiten*, Vol. VI).

Kudicke, R. (1923). Die Blutprotozoen und ihre nächsten Verwandten, pp. 503-520 (Mense, *Handbuch der Tropenkrankheiten*, Vol. IV, Part 2.)

Wenyon, C. M. (1923). Coccidiosis of Cats and Dogs and studies of the *Isospora* of Man. *Ann. Trop. Med. & Parasit.*, Vol. XVII, pp. 231-288.

EXPLANATION OF PLATE V

Figs. 1, 2. Trophozoite inside vacuole.
Figs. 3-8. Various forms of trophozoites.
Fig. 9. Trophozoite inside lymphocyte.
Figs. 10-12. Trophozoites showing nuclear division.
Figs. 13-15. Schizogony.
Fig. 16. A vacuole containing two parasites, a merozoite and one young schizont.
Fig. 17. One cell with two vacuoles each containing a parasite.
Fig. 18. Schizont with four small merozoites.
Fig. 19. Schizont with four large merozoites and a residual body. Note large size of merozoites and residual body.
Figs. 20, 21. Schizonts with eight merozoites.
Fig. 22. Schizont with 13 merozoites showing.
Figs. 23-28. Showing development of macrogamete. Note in 26, 27 and 28, application of nucleus of host cell to wall of vacuole. Note in young macrogametes the large size of the nucleus.
Fig. 29. Macrogamete invaded by a number of microgametes.
Fig. 30. Young microgametocyte.
Fig. 31. Later stage of microgametocyte. Note relationship of the nucleus of host cell to vacuole.
Fig. 32. Later stage of microgametocyte showing mitotic figures and irregular masses of chromatin.
Fig. 33. Still later stage of microgametocyte showing solid masses of chromatin.
Fig. 34. Still later stage of microgametocyte. From each mass of chromatin a small process protrudes.
Fig. 35. Still later stage showing falciform nuclei and one residual body.
Fig. 36. Oöcyst as found in mucosa.
Fig. 37. Oöcyst as found in mucosa and passed in fresh faeces.
Fig. 38. Fully developed oöcyst. (Drawn from material three months' old, hence the residual bodies are small) × 1250.

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